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Dear Joshua:

I said that if I ever obtained any positive results I would write you about them. Until yesterday I have never had any clearly positive results. Even now, there ~~xxx~~ is an alternative explanation that must be ruled out, and I must pile up a larger volume of data, but I think you will agree that the preliminary results are strongly suggestive.

The dual selection method has foundered, as you predicted; iodoacetate resistance seems always to depend on a single locus, closer to threonine than to leucine (but close to both) in your W-677; azide resistance always seems to be closely linked to leucine~~less~~. I still think the method is promising enough to be worth a lot of time looking for the right drugs and strains, but not my time, now. Nisin, a British drug, sounds as good as streptomycin, and might involve two loci; as yet it is practically unobtainable in this country.

Werner, Bernie and I together worked out a logical-enough elaboration of the method you originally used to see if there ~~were~~ more than one recombinant per zygote; this prevents either parent strain from growing. The first full-scale experiment, now in progress, looks very promising.

I superimposed proline-dependence on methionineless in your 58-161 strain, then crossed it with W-677 on the surface of agar containing proline and B<sub>1</sub>. I lifted off 102 of the colonies and plated them individually into agar containing methionine, threonine and leucine, and at the same time tested small samples on test plates containing B<sub>1</sub>, proline, and both. Eight of the colonies gave diffuse growth in the pour-plates and grew on all three test plates. 13 failed to grow out at all. ~~28xxx~~ Nearly all the other ~~pour~~ plates contained scattered (1-50) colonies. Of these, 28 of the strains grew only in the presence of both proline and B<sub>1</sub> on the test plates, so could not have given rise to the colonies by mutation. Mutants from the parental strains should be extremely rare.

On further tests, so far performed on only 7 of the 28 colonies, I have compared the principal type from each colony with the types growing out in the complementary plates. (Evidently lactose and proline~~less~~ are closely linked)

		M	T	L	B <sub>1</sub>	P	Lactose
7 principal types, all		+	+	+	-	-	+
complementary types:	4	-	-	-	+	+	-
(one plate contained	2	-	+	+	+	+	-
two types)	2	+	-	-	+	+	-

The "complementary" types could be independent recombinants that happened to be contained in the colony, although the high frequency of the  $B_1^+ P^+$  type would imply an awful lot of undetected recombinants. If they are segregants from the same zygote, by a process comparable to meiosis, they should exhibit 5 characteristics, mostly statistical:

1. (Treating T and L as one locus) The ratio of  $M-T-L^- : M-T^+ L^+ : M^+ T^- L^-$  (complete prototrophs are eliminated) should be 2:1:1, instead of 1:1:1 as in a random population of  $P^+ B_1^+$ . Linkage effects (mainly  $B_1-M$ ) I can control by seeing how closely a random population of  $P^+ B_1^+$  ~~proteobacteria~~ colonies reproduce this 1:1:1 ratio.
2. Negative correlation between the principals and the complementaries with respect to unselected, loosely-linked markers.
3. When crossing-over occurs in the principal between T and L or between P and Lac, it should frequently occur also in one of the complementaries.
4. Failure to find 3 types of complementaries in a single colony, and very low ~~relative~~ frequency of two types compared with expectation on the hypothesis that they are independent recombinants.
5. When two complementaries are found in a colony, they should never both share a trait of the principal.

All of these will require a fairly extensive body of data, and 4 will require as many as possible dependable markers. I'm sure I haven't yet thought everything through, and I'll welcome any comments or suggestions you want to make. If the next few tests ~~contradict~~ contradict the initial results, I'll let you know soon.

I have made no progress with meningococci--they still don't grow well in broth or on agar--and I may drop them completely now.

With kindest regards for you both,

Sincerely,

*Gordon*